ClinGen Familial Hypercholesterolemia Expert Panel Specifications to the ACMG/AMP Variant Classification Guidelines Version 1.1

This version is specified for the following genes: *LDLR*

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50004
See full pre-print publication: https://doi.org/10.1101/2021.03.17.21252755

Gene	**Disease**	**Transcript**
LDLR	***hypercholesterolemia, familial (MONDO:0007750)***	***NM_000527.5***
PATHOGENIC CRITERIA		
Criteria	**Criteria Description**	***LDLR* Specification**
VERY STRONG CRITERIA		
PVS1	See PVS1 flow diagram (**Figure 1**).	Disease specific / strength
STRONG CRITERIA		
PS1	Missense variant at the same codon as a variant classified pathogenic (by these guidelines), and predicts the same amino acid change. **Caveat**: there is no *in silico* predicted splicing impact for either variant.	Clarification
PS2	Variant is *de novo* in a patient with the disease and no family history. Follow SVI guidance for *de novo* occurrences: https://clinicalgenome.org/working-groups/sequence-variant-interpretation/	Clarification
PS3	Variant meets Level 1 pathogenic functional study criteria. See **Table 3**.	Disease specific / strength
PS4	Variant is found in ≥10 unrelated FH cases (FH diagnosis met by validated clinical criteria). **Caveat**: variant must also meet PM2.	Disease specific / strength
PVS1_Strong	See PVS1 flow diagram (**Figure 1**).	Disease specific / strength
PM5_Strong	Missense variant at a codon with ≥2 missense variants classified pathogenic (by these guidelines), and predicts a different amino acid change.	Strength
PP1_Strong	Variant segregates with phenotype in ≥6 informative meioses in ≥1 family. Must include ≥2 affected relatives (LDL-C >75th centile) with the variant.	Disease specific / strength
MODERATE CRITERIA		
PM1	Missense variant located in exon 4, or a missense change in one of 60 highly conserved cysteine residues (listed in **Supp. Table 4**). **Caveat**: variant must also meet PM2.	Disease specific
PM2	Variant has a PopMax MAF ≤0.0002 (0.02%) in gnomAD. Consider exceptions for known founder variants.	Disease specific
PM3	This criterion can be used for a candidate *LDLR* variant observed in an individual with a homozygous FH phenotype when there is only one other pathogenic variant in *LDLR* (in *trans*), *APOB* or *PCSK9*. **Caveat**: variant must also meet PM2.	Disease specific

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PM4	In-frame deletion/insertions smaller than one whole exon, or in-frame whole-exon duplications not considered in any PVS1 criteria. **Caveat**: variant must also meet PM2.	Disease specific
PM5	Missense variant at the same codon as a variant classified pathogenic (by these guidelines), and predicts a different amino acid change.	Clarification
PM6	See PS2 above.	Clarification
PS3_Moderate	Variant meets Level 2 pathogenic functional study criteria. See **Table 3**.	Disease specific / strength
PS4_Moderate	Variant is found in 6-9 unrelated FH cases (FH diagnosis made by validated clinical criteria). **Caveat**: variant must also meet PM2.	Disease specific / strength
PP1_Moderate	Variant segregates with phenotype in 4-5 informative meioses in ≥1 family. Must include ≥2 affected relatives (LDL-C >75th centile) with the variant.	Disease specific / strength
PVS1_Moderate	See PVS1 flow diagram (**Figure 1**).	Disease specific / strength
SUPPORTING CRITERIA		
PP1	Variant segregates with phenotype in 2-3 informative meioses in ≥1 family. Must include ≥1 affected relative (LDL-C >75th centile) with the variant.	Disease specific / strength
PP2	*Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease.*	*N/A*
PP3	REVEL score ≥0.75 (missense variants), or predicted impact to splicing using MaxEntScan (see **Fig. 2** for suggested thresholds).	Disease specific
PP4	Any *LDLR* variant identified in an FH patient [diagnosis based on validated clinical criteria, e.g. Dutch Lipid Clinic Network (≥6), Simon Broome (possible/definite), MEDPED], **after alternative causes of high cholesterol are excluded**. **Caveat**: variant must also meet PM2.	Disease specific
PP5	*Reputable source recently reports variant as pathogenic but the evidence is not available to the laboratory to perform an independent evaluation.*	*N/A*
PS3_Supporting	Variant meets Level 3 pathogenic functional study criteria. See **Table 3**.	Disease specific / strength
PS4_Supporting	Variant is found in 2-5 unrelated FH cases (FH diagnosis made by validated clinical criteria). **Caveat**: variant must also meet PM2.	Disease specific / strength

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BENIGN CRITERIA		
STAND ALONE CRITERIA		
BA1	Variant has a PopMax FAF ≥0.005 (0.5%) in gnomAD.	Disease specific
STRONG CRITERIA		
BS1	Variant has a PopMax FAF ≥0.002 (0.2%) in gnomAD.	Disease specific
BS2	Variant is identified in ≥3 heterozygous or ≥1 homozygous **well-phenotyped, untreated, normolipidemic** adults (unrelated).	Disease specific
BS3	Variant meets Level 1 benign functional study criteria. See **Table 3**.	Disease specific / strength
BS4	Lack of segregation in ≥2 index case families (unrelated), when data is available for ≥2 informative meioses in each family. **Caveat**: must be ≥1 unaffected relative (LDL-C <50th centile) who is positive for the variant.	Disease specific
SUPPORTING CRITERIA		
BP1	*Missense variant in gene where only LoF causes disease.*	*N/A*
BP2	If a FH patient with a heterozygous phenotype has a proven pathogenic variant in *LDLR (*in *trans), APOB* or *PCSK9*, BP2 is applicable to any additional *LDLR* variants.	Disease specific
BP3	*In-frame deletions/insertions in a repetitive region without a known function.*	*N/A*
BP4	REVEL score ≤0.5 (missense variants), or no predicted impact to splicing using MaxEntScan (see **Fig. 2** for suggested thresholds).	Disease specific
BP5	*Variant found in a case with an alternate molecular basis for disease.*	*N/A*
BP6	*Reputable source recently reports variant as benign but the evidence is not available to the laboratory to perform an independent evaluation.*	*N/A*
BP7	Variant is synonymous. **Caveat**: variant must also meet BP4 (i.e. no predicted impact on splicing).	Disease specific
BS3_Supporting	Variant meets Level 3 benign functional study criteria. See **Table 3**.	Disease specific / strength

Abbreviations: FH, familial hypercholesterolemia; MAF, minor allele frequency; FAF, filtering allele frequency; LoF, loss of function. Note: PopMax refers to the gnomAD subpopulation with the highest allele frequency.

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Supplementary Table 1. Rules for combining pathogenic and benign criteria in ACMG/AMP guideline specifications for *LDLR*.

PATHOGENIC		
1 Very Strong AND	1 or more Strong	
	2 or more Moderate	
	1 Moderate AND	1 Supporting
	2 or more Supporting	
≥2 Strong		
1 Strong AND	3 or more Moderate	
	2 Moderate AND	2 or more Supporting
	1 Moderate AND	4 or more Supporting
LIKELY PATHOGENIC		
1 Very Strong AND	1 Moderate	
1 Strong AND	1-2 Moderate	
	2 or more Supporting	
3 or more Moderate		
2 Moderate AND	2 or more Supporting	
1 Moderate AND	4 or more Supporting	
BENIGN		
1 Stand Alone		
2 or more Strong		
LIKELY BENIGN		
1 Strong AND	1 Supporting	
2 or more Supporting		
Variant of Uncertain Significance (VUS)		
Criteria shown above are not met OR the criteria for pathogenic and benign are contradictory		

Adapted from Richards et al., 2015; no changes to original scoring algorithm.

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PVS1

PVS1 flowchart FH VCEP adaptation

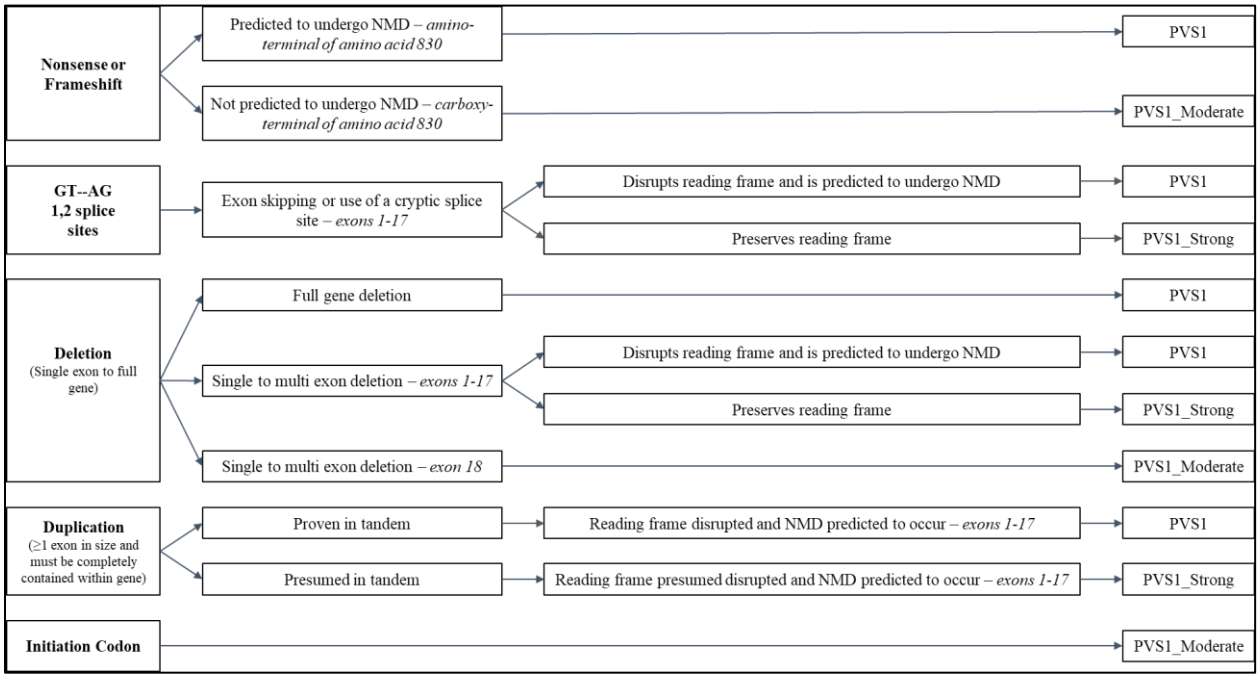

Figure 1. *LDLR*-specific recommendations for application of PVS1. Adapted from Tayoun et al., 2018
Abbreviations: NMD, nonsense-mediated decay

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Supplementary Table 3. *LDLR* exon information.

Exon No.	Start (g.)	Stop (g.)	Start (c.)	Stop (c.)	Length	Start Phase	End Phase
1	11089463	11089615	-86	67	153	-	1
2	11100223	11100345	68	190	123	1	1
3	11102664	11102786	191	313	123	1	1
4	11105220	11105600	314	694	381	1	1
5	11106565	11106687	695	817	123	1	1
6	11107392	11107514	818	940	123	1	1
7	11110652	11110771	941	1060	120	1	1
8	11111514	11111639	1061	1186	126	1	1
9	11113278	11113449	1187	1358	172	1	2
10	11113535	11113762	1359	1586	228	2	2
11	11116094	11116212	1587	1705	119	2	1
12	11116859	11116998	1706	1845	140	1	0
13	11120092	11120233	1846	1987	142	0	1
14	11120370	11120522	1988	2140	153	1	1
15	11123174	11123344	2141	2311	171	1	1
16	11128008	11128085	2312	2389	78	1	1
17	11129513	11129670	2390	2547	158	1	0
18	11131281	11133820	2548	2583	35	0	-

Phase: the position of an exon/intron boundary within a codon. A phase of zero means the boundary falls between codons, one means between the first and second base and two means between the second and third base. Genomic (g.) cooridinates correspond to reference sequence NC_000019.9, and coding (c.) cooridinates correspond to *LDLR* transcript NM_000527.5.

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PS3, BS3

Functional study criteria specifications for *LDLR*.

Table 3. PS3/BS3 functional study criteria specifications for *LDLR*.

Pathogenic	
PS3 (Level 1)	**(1)** Study of the *whole* LDLR cycle (LDLR expression/biosynthesis, LDL binding, and LDL internalization) performed in heterologous cells (with no endogenous LDLR) transfected with mutant plasmid. Assay result of <70% of wild-type activity in either expression/biosynthesis, binding OR internalization.
PS3_Moderate (Level 2)	**(1)** Study of a) only *part* of the LDLR cycle following Level 1 methodology, or b) *whole or part* of the LDLR cycle in true homozygous patient cells. A variant with assay results of <70% of wild type activity in either LDLR expression/biosynthesis, LDL binding OR internalization. **(2)** RNA studies, using RNA extracted from heterozygous or true homozygous patient cells, where aberrant transcript is confirmed by sequencing and is quantified as >25% of total transcript from heterozygous cells or 50% of total transcript from homozygous cells. **(3)** Variants with two or more Level 3 functional studies (must be different assays); or any Level 3 functional study #1-4 performed by two or more independent labs with concordant results.
PS3_Supporting (Level 3)	**(1)** Study of LDLR cycle (*whole or part*) in heterozygous patient cells, with assay results of <85% of wild-type activity in either LDLR expression/biosynthesis, LDL binding OR internalization. **(2)** Luciferase studies with transcription levels of <50% compared to wild-type (applicable to 5'UTR/promoter variants). **(3)** Minigene splicing assays with <10% wild-type transcript present where an aberrant transcript from the candidate variant is confirmed by sequencing. **(4)** High-throughput assays, which include alternative microscopy assays (e.g., Thormaehlen et al., 2015), Multiplex Assays of Variant Effect (MAVE) (e.g., Weile & Roth, 2018) and deep mutational scanning assays, can be considered

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	here, only if assay has been validated with a minimum of four pathogenic and four benign variant controls in LDLR. *Note: % activity thresholds will be defined by the FH VCEP as more data becomes available. **(5)** RNA studies, using RNA extracted from heterozygous or homozygous patient cells, with aberrant transcript confirmed by sequencing (but without transcript quantification).
Benign	
BS3 (Level 1)	**(1)** Study of the *whole* LDLR cycle (LDLR expression/biosynthesis, LDL binding, and LDL internalization) performed in heterologous cells (with no endogenous LDLR) transfected with mutant plasmid. Assay result of >90% of wild-type activity in expression/biosynthesis, binding AND internalization. Note: studies of only part of the LDLR cycle are not eligible for BS3 or BS3_Supporting.
BS3_Supporting (Level 3)	**(1)** Study of *whole* LDLR cycle in a) true homozygous patient cells, with assay result of >90% of wild-type activity in biosynthesis, binding AND internalization; or in b) heterozygous patient cells with assay result of >95% of wild-type activity in biosynthesis, binding AND internalization. **(2)** Luciferase studies with transcription levels of >90% when compared to wild-type (applicable to 5'UTR/promoter variants). **(3)** RNA studies, using RNA extracted from heterozygous or homozygous patient cells, with a) aberrant transcripts quantification, where aberrant transcript is <10% of total transcript OR b) without transcript quantification where no aberrant transcript is confirmed by sequencing. **(4)** Minigene splicing assay where only wild-type transcript is present and confirmed by sequencing. **(5)** High-throughput assays as defined above; only applicable when assay can indicate the *whole* LDLR cycle (LDLR expression/biosynthesis, LDL binding AND internalization) is unaffected.

Note: functional assays performed in compound heterozygous patient cells are not considered applicable in PS3/BS3 criteria since it is difficult to delineate the individual effect of each variant.

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PM1

Supplementary Table 4. *LDLR* cysteine residues involved in disulfide bond formation.

Residue	Domain	Structure analysis	Predicted impact on LDLR structure and/or function
p.Cys27	LDL-receptor class A 1	disulfide bond	folding defect
p.Cys34	LDL-receptor class A 1	disulfide bond	folding defect
p.Cys39	LDL-receptor class A 1	disulfide bond	folding defect
p.Cys46	LDL-receptor class A 1	disulfide bond	folding defect
p.Cys52	LDL-receptor class A 1	disulfide bond	folding defect
p.Cys63	LDL-receptor class A 1	disulfide bond	folding defect
p.Cys68	LDL-receptor class A 2	disulfide bond	folding defect
p.Cys75	LDL-receptor class A 2	disulfide bond	folding defect
p.Cys82	LDL-receptor class A 2	disulfide bond	folding defect
p.Cys89	LDL-receptor class A 2	disulfide bond	folding defect
p.Cys95	LDL-receptor class A 2	disulfide bond	folding defect
p.Cys104	LDL-receptor class A 2	disulfide bond	folding defect
p.Cys109	LDL-receptor class A 3	disulfide bond	folding defect; LDL binding defect
p.Cys116	LDL-receptor class A 3	disulfide bond	folding defect; LDL binding defect
p.Cys121	LDL-receptor class A 3	disulfide bond	folding defect; LDL binding defect
p.Cys128	LDL-receptor class A 3	disulfide bond	folding defect; LDL binding defect
p.Cys134	LDL-receptor class A 3	disulfide bond	folding defect; LDL binding defect
p.Cys143	LDL-receptor class A 3	disulfide bond	folding defect; LDL binding defect
p.Cys148	LDL-receptor class A 4	disulfide bond; acidic pH intramolecular binding interface	folding defect; receptor-recycling defect; LDL binding defect
p.Cys155	LDL-receptor class A 4	disulfide bond	folding defect; LDL binding defect
p.Cys160	LDL-receptor class A 4	disulfide bond; acidic pH intramolecular binding interface	folding defect; receptor-recycling defect; LDL binding defect
p.Cys167	LDL-receptor class A 4	disulfide bond	folding defect; LDL binding defect
p.Cys173	LDL-receptor class A 4	disulfide bond; acidic pH intramolecular binding interface	folding defect; receptor-recycling defect; LDL binding defect
p.Cys184	LDL-receptor class A 4	disulfide bond	folding defect; LDL binding defect
p.Cys197	LDL-receptor class A 5	disulfide bond	folding defect; LDL binding defect
p.Cys204	LDL-receptor class A 5	disulfide bond	folding defect; LDL binding defect
p.Cys209	LDL-receptor class A 5	disulfide bond	folding defect; LDL binding defect
p.Cys216	LDL-receptor class A 5	disulfide bond	folding defect; LDL binding defect

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p.Cys222	LDL-receptor class A 5	disulfide bond; acidic pH intramolecular binding interface	folding defect; receptor-recycling defect; LDL binding defect
p.Cys231	LDL-receptor class A 5	disulfide bond	folding defect; LDL binding defect
p.Cys236	LDL-receptor class A 6	disulfide bond	folding defect; LDL binding defect
p.Cys243	LDL-receptor class A 6	disulfide bond	folding defect; LDL binding defect
p.Cys248	LDL-receptor class A 6	disulfide bond	folding defect; LDL binding defect
p.Cys255	LDL-receptor class A 6	disulfide bond	folding defect; LDL binding defect
p.Cys261	LDL-receptor class A 6	disulfide bond	folding defect; LDL binding defect
p.Cys270	LDL-receptor class A 6	disulfide bond	folding defect; LDL binding defect
p.Cys276	LDL-receptor class A 7	disulfide bond	folding defect; LDL binding defect
p.Cys284	LDL-receptor class A 7	disulfide bond	folding defect; LDL binding defect
p.Cys289	LDL-receptor class A 7	disulfide bond	folding defect; LDL binding defect
p.Cys296	LDL-receptor class A 7	disulfide bond	folding defect; LDL binding defect
p.Cys302	LDL-receptor class A 7	disulfide bond	folding defect; LDL binding defect
p.Cys313	LDL-receptor class A 7	disulfide bond	folding defect; LDL binding defect
p.Cys318	EGF-like 1	disulfide bond	folding defect; LDL binding defect
p.Cys325	EGF-like 1	disulfide bond	folding defect; LDL binding defect
p.Cys329	EGF-like 1	disulfide bond	folding defect; LDL binding defect
p.Cys338	EGF-like 1	disulfide bond	folding defect; LDL binding defect
p.Cys340	EGF-like 1	disulfide bond	folding defect; LDL binding defect
p.Cys352	EGF-like 1	disulfide bond	folding defect; LDL binding defect
p.Cys358	EGF-like 2; calcium-binding	disulfide bond	folding defect; receptor-recycling defect
p.Cys364	EGF-like 2; calcium-binding	disulfide bond	folding defect; receptor-recycling defect
p.Cys368	EGF-like 2; calcium-binding	disulfide bond	folding defect; receptor-recycling defect
p.Cys377	EGF-like 2; calcium-binding	disulfide bond	folding defect; receptor-recycling defect
p.Cys379	EGF-like 2; calcium-binding	disulfide bond	folding defect; receptor-recycling defect
p.Cys392	EGF-like 2; calcium-binding	disulfide bond	folding defect; receptor-recycling defect
p.Cys667	EGF-like 3	disulfide bond	folding defect; receptor-recycling defect
p.Cys677	EGF-like 3	disulfide bond	folding defect; receptor-recycling defect
p.Cys681	EGF-like 3	disulfide bond	folding defect; receptor-recycling defect
p.Cys696	EGF-like 3	disulfide bond	folding defect; receptor-recycling defect
p.Cys698	EGF-like 3	disulfide bond	folding defect; receptor-recycling defect
p.Cys711	EGF-like 3	disulfide bond	folding defect; receptor-recycling defect

Adapted from Guo et al., 2019. Residues correspond to *LDLR* transcript NM_000527.5.
Abbreviations: Cys, cysteine; EGF, epidermal growth factor

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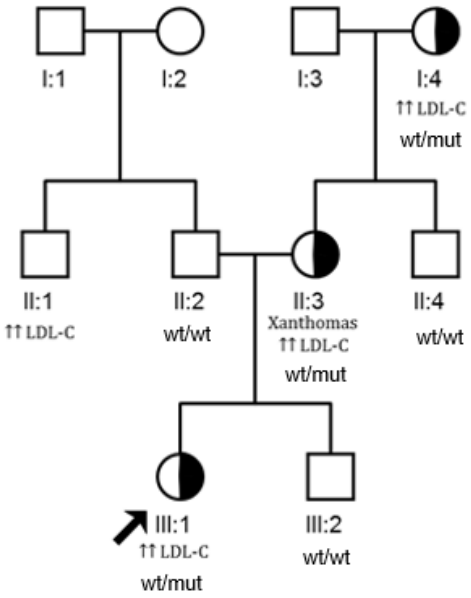
PP1, BS4

Pedigree of a FH family.



Supplementary Figure 2. Pedigree of a FH family.
Index case is identified with an arrow. Half-filled symbols represent heterozygous individuals.
Index case III:1 inherited her *LDLR* variant from the maternal (II:3) side of the family. Her father (II:2) has normal cholesterol, no cardiovascular disease history, and is negative for the *LDLR* variant; therefore, her father (II:2) and paternal uncle (II:1) should not be considered in the co-segregation study. Similarly, the maternal grandfather (I:3) should not be considered.

In this family the individuals that can be considered informative meioses are the index case's brother (III:2), mother (II:3), maternal uncle (II:4) and maternal grandmother (I:4).

Index cases should not be counted as positive cases for co-segregation results.

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BA1, BS1, PM2

LDLR-specific population data frequency thresholds.

Table 2. *LDLR*-specific population data frequency thresholds.

	gnomAD Frequency	Prevalence	Penetrance	Allelic Het.	Genetic Het.
BA1	PopMax FAF ≥0.005 (0.5%)[a]	1/250	50%	1.0	1.0
BS1	PopMax FAF ≥0.002 (0.2%) and <0.005 (0.5%)	1/250	95%	1.0	0.9
PM2	PopMax MAF ≤0.0002 (0.02%)	1/250	95%	0.1	0.9

Note: PopMax refers to the gnomAD subpopulation with the highest allele frequency. [a] BA1 metrics were equal to 0.4%; however, we conservatively increased the BA1 threshold to 0.5%. Abbreviations: FAF, filtering allele frequency; MAF, minor allele frequency; Het., heterogeneity.

PP3, BP4

Do not apply if PVS1 (or modified strength) is met.
If both "missense" and splicing prediction are applicable, only 1 prediction of affecting function is necessary to apply PP3, but both need to predict a benign effect for BP4 to be given.

In silico classification of missense variants in *LDLR*

We recommend the use of REVEL,

a) scores above 0.75 as supportive evidence of pathogenicity (PP3),

b) scores below 0.50 as supportive evidence of benign (BP4).

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In silico prediction of splicing effects in *LDLR*

Do not apply if *splicing* functional data is available.
Apply A, B or C based on variant location and use MaxEntScan (MES) for scores:

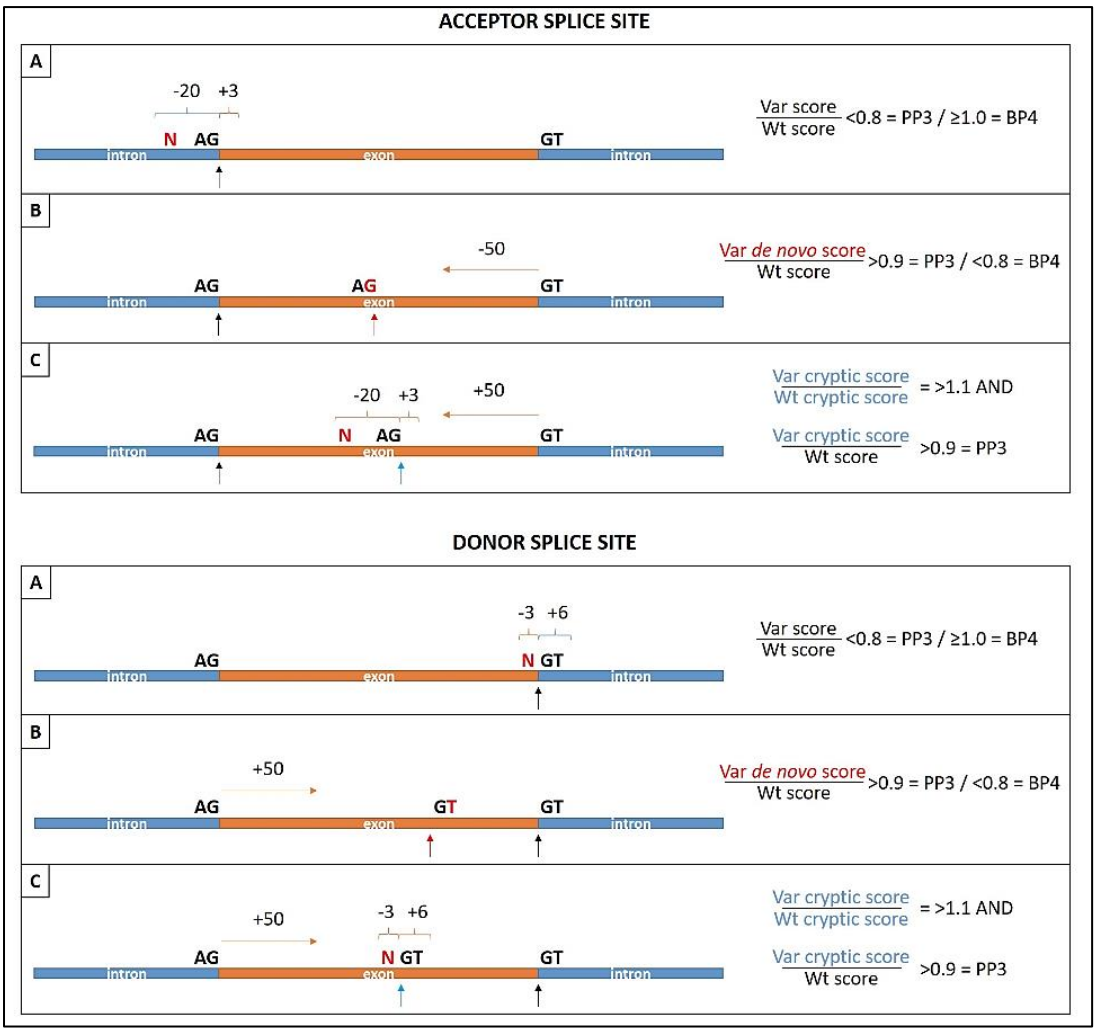

Figure 2. FH VCEP suggestions for evaluating splicing effects using MaxEntScan (MES) dependent on variant location A, B, or C.

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(**A**) Variant is located at -20 to +3 bases related to the authentic acceptor splice site or at -3 to +6 related to the authentic donor splice site: A result of authentic splice site strength variant/wild-type score <0.8 is supportive evidence of pathogenicity (PP3), while a score ≥1.0 is supportive evidence of benign (BP4).

(**B**) Variant creates *de novo* acceptor splice site, which is at least 50 bases upstream of the authentic donor splice site, or *de novo* donor splice site, which is at least 50 bases downstream of the authentic acceptor splice site: A result of *de novo* splice site strength variant/authentic wild-type score in >0.9 is applicable to PP3, while a score <0.8 is applicable to BP4.

(**C**) Variant is located at -20 to +3 bases relative to an intra-exonic AG dinucleotide, which is at least 50 bases upstream of the authentic donor splice site, or at -3 to +6 bases relative to an intra-exonic GT dinucleotide, which is at least 50 bases downstream of the authentic acceptor splice site: Results of both variant cryptic/wild-type cryptic score in >1.1 and cryptic acceptor/authentic acceptor score or cryptic donor/authentic donor score in >0.9 is applicable to PP3.

Note: BP4 is applicable to exonic variants outside of the 50 base limits detailed above, given the unlikelihood of such variants to impact splicing in *LDLR*.

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